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REVIEW

γH2AX: a sensitive molecular marker of DNA damage and repair

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Phosphorylation of the Ser-139 residue of the histone variant H2AX, forming γH2AX, is an early cellular response to the induction of DNA double-strand breaks. Detection of this phosphorylation event has emerged as a highly specific and sensitive molecular marker for monitoring DNA damage initiation and resolution. Further, analysis of γH2AX foci has numerous other applications including, but not limited to, cancer and aging research. Quantitation of γH2AX foci has also been applied as a useful tool for the evaluation of the efficacy of various developmental drugs, particularly, radiation modifying compounds. This review focuses on the current status of γH2AX as a marker of DNA damage and repair in the context of ionizing radiation. Although the emphasis is on γ-radiation-induced γH2AX foci, the effects of other genotoxic insults including exposure to ultraviolet rays, oxidative stress and chemical agents are also discussed.
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Introduction

Double-strand breaks (DSBs) are considered to be among the most lethal forms of DNA damage, severely compromising genomic stability.[1,2] They form as a result of two single-stranded nicks in opposing DNA strands occurring sufficiently close to one another, typically within 10–20 base pairs.[3,4] It is critical that DSBs are repaired quickly and precisely to avoid cellular death, chromosomal aberrations, mutations and in certain cases initiation of pathological effects such as cancer. DNA damage-associated histone modifications have been in the spotlight over the last few years as these modifications are important in providing DNA repair factors access to damaged DNA.[5]

An early cellular response to DSBs is the rapid phosphorylation of H2AX, the minor histone H2A variant, at mammalian Ser-139 to produce γH2AX.[6] The formation of γH2AX was first discovered in yeast where non-homologous end joining was impaired with the loss of the C-terminus of H2A, which contained Ser-129, the yeast homologue of mammalian Ser-139. This phosphorylation event is now one of the most well-established chromatin modifications linked to DNA damage and repair.[7]

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In recent years, immunofluorescence based assays that allow the visualization of discrete nuclear foci formed as a result of H2AX phosphorylation have emerged as very sensitive and reliable methods of detecting DSBs. Quantification of individual γH2AX foci by fluorescence microscopy has become the preferred method of DSB detection given that each break has been found to correspond to one γH2AX focus.[7,8] DSB detection based on γH2AX foci is 100-fold or more sensitive than other methods of detecting DSBs at clinically relevant doses, enabling measurements of radiation doses as low as 1 mGy, which corresponds to one focus in every 30 cells.[9,10] As γH2AX is formed *de novo*, it is a more reliable DSB marker than other repair proteins that are present in cells even when DNA is not damaged.[11] γH2AX foci detection allows the distinction of the temporal and spatial distribution of DSB formation unlike previous methods such as constant field gel electrophoresis or pulsed field gel electrophoresis, which only detect DSBs in the nucleus after large doses of ionizing radiation (IR) (5–50 Gy), which are typically well above biologically relevant doses.[12,13] Additionally, in contrast to the Comet assay, analysis of γH2AX foci does not require lysis at high temperatures.[14]

This aim of this review is to address the current status of γH2AX as a marker of DNA damage and repair, particularly after IR, with an emphasis on X-rays and γ-radiation. Phosphorylation of γH2AX in response to other genotoxic insults including UV exposure, oxidative species and other chemical agents is also discussed.

Role of γH2AX in DNA DSB signalling and repair

Immediate and efficient error correction after DSB induction is important to restore and preserve chromatin architecture. A series of complex DNA pathways collectively known as the DNA damage response (DDR) is responsible for the recognition, signalling and repair of DSBs in cells.[15] Cytogenic and mutagenic properties of DSBs are overcome by two major mechanisms of DSB repair, namely, homologous recombination and non-homologous end joining, each with distinct and overlapping roles in maintaining genomic integrity. The type of organism, phase of the cell cycle, cell type, stage of development and complexity of the break have a role in dictating which pathway a cell will take on encountering a DSB.[16]

Non-homologous end joining is an error-prone DSB repair pathway, typically used after IR-induced damage in mammalian cells.[17] Briefly, Ku70/80 and DNA-protein kinase catalytic subunit (DNA-PKcs) are responsible for DSB identification and the protection of DNA ends whereas Artemis carries out

end-processing to facilitate ligation by the XRCC4-Ligase IV complex.[16,18,19]

The second major pathway used to restore the integrity and functionality of chromatin is homologous recombination, the efficient, error-free alternative for DNA repair. Homologous recombination restores chromatin integrity without loss of genetic information using an unaffected complementary DNA strand as a template.[19–21] As such, this pathway can only be used after DNA replication in the S and G2 phases of the cell cycle where a homologous chromosome is present.[16] In this pathway, initial DNA end-processing is carried out by nucleases that resect the damaged DNA ends in the 5′–3′ direction followed by strand extension on detection of an undamaged homologous DNA template.[22,23] DNA crossovers, also known as Holliday junctions, are resolved after branch migration, generating two complete DNA molecules.[3,24]

H2AX phosphorylation is a key step in the DDR, playing a role in signalling and initiating the repair of DSBs[9] (Figure 1). The contribution of γH2AX to signalling is thought to lie in the ability of this chromatin modification to increase DNA accessibility, leading to the recruitment and accumulation of specific DDR proteins at DNA ends.[12,25] H2AX phosphorylation creates an epigenetic signal that is recognized by specific domains on downstream DDR proteins.[26] Further, γH2AX facilitates DSB rejoining by anchoring broken ends together through nucleosome repositioning at damaged sites and a reduction in chromatin density.[27–29] Cohesins are recruited by γH2AX to keep ends in close proximity during repair, preventing the loss of large chromosomal regions.[30,31] In addition, γH2AX modulates checkpoint responses, giving DNA time to repair.[32] Surprisingly, this appears to be true only at relatively low levels of DNA damage[12] suggesting that alternative pathways may be engaged when cells encounter higher levels of damage.[3]

Although γH2AX may not be a critical component of either the mammalian homologous recombination or non-homologous end joining repair pathways, it has a role in the repair of a subset of DSBs, increases the efficiency of DNA repair and reduces radiosensitivity.[2,12,33,34] H2AX null mice are extremely radiosensitive and have aberrant genomes, require longer DNA damage repair times, have high a incidence of aberrant metaphases as well as defective G2/M cell cycle kinetics.[2,35]

Temporal and spatial distribution of IR-induced γH2AX

The effect of X-rays and γ-radiation on γH2AX foci formation has been widely documented in the literature. On exposure to IR, DSBs are induced, leading to local phosphorylation at the highly conserved SQEY motif of H2AX, which is a common substrate for the phosphatidyl-inosito-3 kinase (PIKK) family of proteins that include the key DSB repair proteins, ataxia telangiectasia mutated (ATM), DNA-PKcs and ATM and RAD3-related (ATR).[36,37] Direct phosphorylation is initiated at DSB sites.[38,39] Although only a few base pairs may be implicated in the DSB itself, there is a significant signal amplification, highlighting the importance of chromatin modifications to initiate signalling and repair pathways.[14,40] γH2AX spreads to adjacent areas of chromatin, affecting ~0.03% of

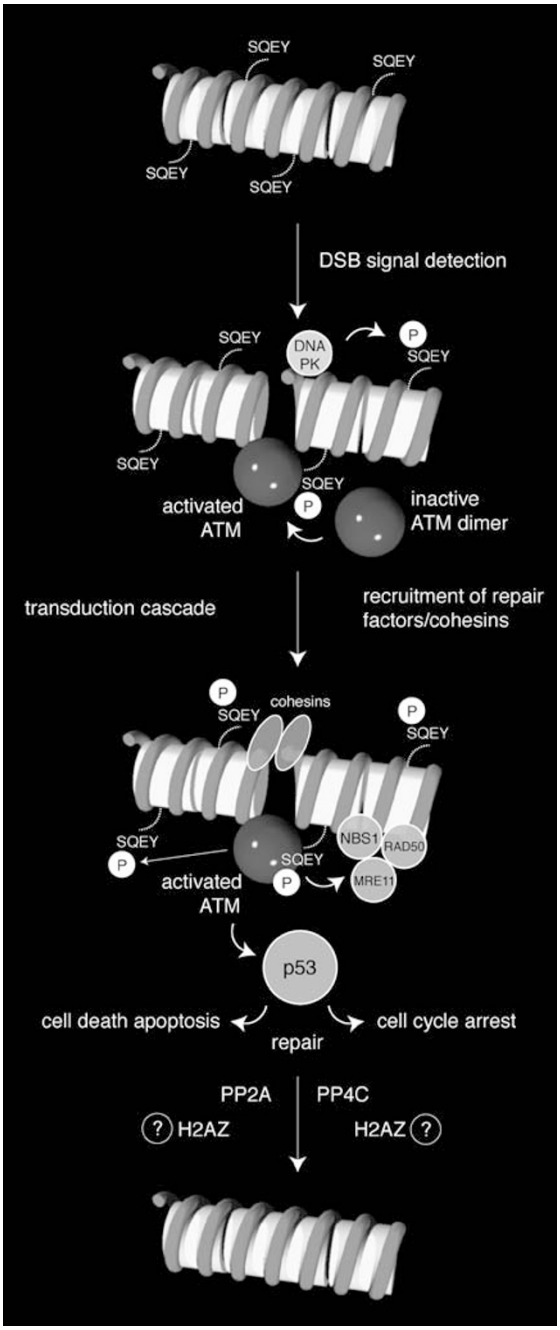

Figure 1 Simplified schematic representation of molecular responses to DSBs. The overall DSB response is typically considered as a classical signal transduction cascade. Phosphorylation of H2AX (nucleosomes consisting of H2AX are depicted with the highly conserved SQEY tail) is a key step in signalling and initiating the repair of DSBs. H2AX is phosphorylated at Ser-139 by the protein kinase ATM, which is activated in response to DSBs by autophosphorylation of Ser-1981 residues in inactive dimers. The PIKK kinases DNA-PKcs and ATR (predominantly in response to UV-mediated DSBs) also phosphorylate H2AX. After the initial phosphorylation of H2AX at sites flanking DSBs, numerous DSB repair proteins including the MRN complex and cohesins, which maintain the DNA ends in close proximity during repair, are recruited. This leads to further activation of ATM and phosphorylation of H2AX histones, forming γH2AX foci that are easily detected by immunofluorescence-based assays. The activated ATM monomers phosphorylate numerous downstream targets including p53 to mediate cellular responses, which may include apoptosis and cell death, cell cycle arrest or repair. It has been proposed that after repair γH2AX is removed by dephosphorylation by the phospahatases PP2A and PP4C. However, redistribution of γH2AX in chromatin by acetyltransferase-mediated histone exchange, replacing γH2AX with H2AZ, has also been suggested.

total cellular H2AX per DSB, corresponding to ~2000 H2AX molecules within 2 Mbp of chromatin.[40,41] Interestingly, immunofluorescence staining has revealed that regions up to 30 Mbp may be modified. This suggests that massive chromatin relaxation takes place and phosphorylation may not occur at every neighbouring H2AX molecule given that H2AX is thought to be present in distinct clusters, rather than being evenly distributed throughout the chromosome.[2,8,42]

As mentioned above, ATM, DNA-PKcs and ATR are the three main kinases involved in H2AX phosphorylation. Each kinase has a specific role in phosphorylating H2AX and activating other repair and signalling factors to regulate a myriad of cellular processes, which will ultimately result in either cell survival or cell death.[43] These PIKK kinases have overlapping substrate specificities and function in parallel, however ATM, the main kinase responsible for γH2AX phosphorylation under normal physiological conditions, primarily mediates IR-induced DNA damage signalling with some redundancy that can be compensated by DNA-PK, whereas UV-induced DNA damage associated with metabolic stress is largely ATR dependent.[6,44–48] The extent of damage because of IR, UV and endogenous DSB inducers differs greatly as IR is a more potent DSB inducer, and this difference may indicate different types of DSBs, which may well be the determinant of the kinase summoned.[49] γH2AX is induced by both high and low linear energy transfer radiation and the number of DSBs induced and damage complexity increases with increasing linear energy transfer.[50] High linear energy transfer exposure leads to greater induction and persistence of γH2AX in cells, as well as larger foci, which may be attributed to further γH2AX formation occurring away from the initial DSB.[51,52]

Several mechanisms have been proposed to explain the origin of H2AX phosphorylation. The first involves direct ATM activation that triggers rapid auto-phosphorylation of Ser-1981 on detecting conformational changes in DNA as a result of local chromatin modifications.[12,53] Another theory allows for the possibility that ATM does not associate directly with DNA but rather is dependent on initial damage detection by the MRN Mre11, Rad50 and Nbs1 (MRN) complex that comprises meiotic recombination 11 (MRE11), RAD50 and nibrin or Nijmegen breakage syndrome 1.[50] MRE11 of the MRN complex is responsible for direct binding to damaged DNA, recruiting ATM that in turn undergoes auto-phosphorylation and monomerization, leading to further recruitment and phosphorylation of distal targets such as H2AX.[54,55] A recent study highlights the importance of activated ATM and mediator of DNA damage checkpoint 1 (MDC1) in regulating the continued local phosphorylation of H2AX along broken DNA strands.[56]

Discrete nuclear foci that form as a result of H2AX phosphorylation are now widely used as a quantitative marker of individual DSBs.[8] These foci are termed IR-induced nuclear foci when they are caused by ionizing radiation.[6] γH2AX foci can be detected using immunofluorescence microscopy even at low quantities just minutes after of γ-radiation, reaching a peak at 30 min post irradiation.[2,57] After a short plateau phase of approximately 1 h, the number of foci starts to decrease with a half-life of several hours.[58] Foci formation has been observed to have a strong linear-dose relationship with a dose threshold of 0.05 Gy in human tissue.[59,60]

Recent studies have suggested the potential of γH2AX quantification for detection of precancerous lesions, as the marker may reflect cancer-associated genomic instability in nuclei.[61–63] Further, γH2AX formation has the potential to act as an indicator of cellular radiosensitivity that may be used to predict individual responses to IR in clinical settings.[64,65] Many cancer treatments induce DSBs as part of the therapeutic effect, and detection of γH2AX foci will indicate the efficacies of therapy as well as allow of proper planning of treatment for each individual patient.[63,66] Furthermore, DSBs are generated in response to genotoxic stress, enabling the use of γH2AX foci as a pharmacodynamic biomarker in the development of anti-cancer drugs and as an indicator of response in patients.[67,68] Foci formation can also potentially act as a relevant biodosimeter for human exposure to IR in cases of unintentional radiation exposure or after radiation therapy in cancer patients. Detection of γH2AX foci is a minimally invasive method that only requires the collection of peripheral blood lymphocytes or skin biopsies.[62,69] In addition, γH2AX foci have been proposed as prospective biomarkers of aging given the accumulation of DSBs observed in senescing cells.[60]

Studies have found that each DSB corresponds to one γH2AX focus but the reverse is not applicable as γH2AX may persist even after DSBs are rejoined.[7,13,14] The discovery of γH2AX foci at DSB sites permits the study of the cellular mechanisms of DDR after DNA damage.[70] Each γH2AX focus functions to alter chromatin structure to increase accessibility and acts as a stage for the accumulation of DDR and repair factors.[65,71,72] Complexes consisting of other DNA repair and signalling molecules such as 53BP1, BRCA1, Nijmegen breakage syndrome 1, RAD50 and RAD51 also form nuclear foci that have been found to co-localize with γH2AX foci.[6,73] The accumulation of these proteins depend on γH2AX phosphorylation but may not accumulate at the same rate.[13] These proteins may be recruited either because of their high affinity for γH2AX or because of changes in chromatin conformation induced by the phosphorylation step.[74] Along with the accumulation of DSB response and repair factors, a small fraction of ATM that is responsible for H2AX phosphorylation is retained in the nucleus and is found to associate with γH2AX foci.[61]

High numbers of small foci are formed at the early stages of the DDR, decreasing in number and increasing in size as the DDR progresses.[75] If the number of DSBs exceeds the cellular capacity, γH2AX are visualized as diffuse halos rather than distinct foci.[61] ATR-dependent γH2AX phosphorylation as a result of UVC radiation does not present as foci but instead, as a diffuse pattern known as pan-nuclear staining.[76] This phenomenon is also observed after TNF-related apoptosis-inducing ligand-induced apoptosis.[77] Global γH2AX has also been observed during mitosis in some cells[78] and the X-Y chromosome pair (sex-body) is completely covered with γH2AX during spermatogenesis.[79] Further, γH2AX has been discovered in the centrosome, which is apparently devoid of DNA.[13]

The loss of γH2AX at DSB sites is thought to reflect the completion of repair of DNA at break sites.[27] It is unclear when a DSB is completely repaired. Some studies suggest that this is on rejoining of both DNA strands whereas others propose that chromatin has to be returned to its original state of compaction before a DSB is truly repaired.[12,80] The way in which γH2AX is removed from chromatin has yet to be completely resolved and several mechanisms have been proposed for the reinstatement of chromatin. The first mechanism involves the dephosphorylation of γH2AX by phosphatases, namely phosphatase 2A and phosphatase 4C.[81–85] The second mechanism of γH2AX removal is through redistribution in the chromatin, which involves histone exchange, replacing γH2AX with H2AZ during chromatin remodelling mediated by the histone acetyltransferases.[86,87] As γH2AX is detectable megabases away from DSB sites, the removal of γH2AX may involve a combination of chromatin remodelling and histone exchange at break sites and γH2AX dephosphorylation at distal sites. Once repaired, γH2AX

is removed to prevent further sequestration of DDR and repair factors.[86] Regions of chromatin affected by DSB repair are restored to their original structure and cell cycle resumes, ensuring both genetic and epigenetic information is preserved.[88]

γH2AX loss has been found to correlate to repair activity only at relatively low levels of DNA damage, typically below 150 DSBs per genome and only in cells proficient in DNA repair.[27,64] At higher doses of IR, early reductions in foci number and intensity are not reflected in the global chromatin signal that remains largely unchanged.[89,90] The global γH2AX expression does not seem to be affected, indicating that reduction in foci numbers may be counterbalanced by larger foci to produce an unchanged γH2AX signal. Another possibility is that increased phosphatase activity at break sites preferentially dephosphorylates γH2AX within foci and reduces the number of DSBs but not the total γH2AX signal.[27] Moreover, chromatin remodelling complexes that replace γH2AX with H2AZ by altering chromatin structure to enhance DNA accessibility and DNA repair may be a factor that contributes to the quicker disappearance of DSBs but not foci.[86]

A striking observation is that heterochromatic sites seem to be resistant to IR-induced DSBs and γH2AX foci are rarely detected in heterochromatin although the region contains a high amount of DNA.[91–93] Epigenetic processes appear to be the best explanation for the exclusion of γH2AX in heterochromatin, however, further mechanistic insights are required. Another characteristic feature of γH2AX generation is chromatin relaxation, resulting in foci formation regardless of the phase in the cell cycle. H2AX phosphorylation not only occurs at interphase but also during the mitotic phase where chromatin is more condensed. However, the difference between the two phases is only apparent in the dephosphorylation step, which is much slower in mitotic cells.[94]

γH2AX and the bystander effect

The bystander effect is a phenomenon that is yet to be fully understood but can be defined as alterations in unexposed cells because of IR exposure of nearby cells.[95,96] The radiation-induced bystander effect is caused by the indirect exposure of non-irradiated cells because of proximity with irradiated cells, leading to biological effects such as altered gene expression, chromosomal aberrations, mutagenesis as well as a reduction in cell survival.[97] The bystander effect is tissue specific, transducing and co-ordinating adaptive responses to either switch the effect on or off but no true dose–response has been documented as yet.[98,99] Among the possible outcomes are apoptosis, mutation fixation and cellular transformation. In contrast with the conventional assumption that cellular death is detrimental, it may instead be beneficial in that it removes damaged cells from the population to reduce the risk of passing on mutations to the next generation.[99]

Recently, DNA DSBs have been established to be another manifestation of the bystander effect that makes γH2AX a potentially useful tool for investigating this effect.[100] Bystander cells have been shown to have increased γH2AX foci indicating the presence DSBs.[101] Evidence of this phenomenon was supported by the observation of other DDR and DNA repair proteins co-localize with γH2AX foci in the bystander cells.[102] γH2AX foci generation in bystander cells differs from that of directly irradiated cells and has been observed to be ATR dependent and predominantly occurs during S phase.[100] Similarly, the DDR repair kinetics also appear to differ from cells directly exposed to IR.[101,103]

A distinct relationship between the distance of bystander cells and IR exposed cells has been defined, that is, bystander cells in closer proximity to IR-exposed cells corresponded to higher incidence of DNA damage compared with cells located further away.[102] This may be due to signal propagation between bystander cells either through gap junctions or by released factors such as pro-inflammatory cytokines.[101,104] The underlying cause of DSBs in bystander cells is thought to be due to the activity of reactive oxygen species (ROS), in particular, nitric oxide that influences signal propagation and damages DNA through base amination, nitration and lipid peroxidation.[105–107] This presents a potential application of γH2AX as an indicator of conditions that are characterized by amplified ROS; an area that warrants further exploration.

γH2AX formation in response to other genotoxic insults

Genotoxic insults such as ultraviolet light (UV) exposure, drugs, chemicals, and endogenous DNA processes can lead to DSBs. Compared with IR, γH2AX generation after UV radiation has not been as widely documented. UV rays cause both direct and indirect DNA damage.[61,108] UVB is regarded as the most cytotoxic form of UV radiation, and is capable of causing direct phototoxicity on absorption of its photons by DNA bases, typically during early S phase.[108,109] S-phase γH2AX generation, which is largely mediated by ATR, reflects DSBs that form because of replication stress when UVB-induced lesions collide with replication forks. The formation of γH2AX in other phases or the cell cycle is possibly a reaction to the initial cellular response to UVB-included DSBs or their repair.[109–112] The indirect mechanism of UV-induced damage involves oxidative stress caused by an imbalance between ROS production and ability of cells to scavenge the reactive intermediates involves ROS production which in turn induces DSBs.[61,113]

Anti-cancer drugs such as DNA replication inhibitors, cross-linking agents and topoisomerase inhibitors are capable of inducing DSBs, which subsequently lead to γH2AX formation because of replication and transcriptional stresses. DNA replication inhibitors, such as hydroxyurea, inhibit DNA replication by affecting deoxynucleotide pools, causing stress at stalled replication forks, which induces DNA damage.[111] Interstrand crosslinking cancer chemotherapeutic agents, such as cisplatin, generate γH2AX indirectly through DNA alkylation at damaged replication forks.[114–116] In addition, DNA topoisomerase I and II inhibitors impede DNA replication, causing replication stress and promoting DSBs through the collision of replication forks.[117,118] DNA topoisomerase I inhibitors including doxorubicin and camptothecin induce H2AX phosphorylation predominantly in S-phase cells whereas DNA topoisomerase II inhibitors, such as etoposide and mitoxantrone, generate γH2AX in all phases of the cell cycle, especially during G1.[119,120] H2AX phosphorylation induced by these drugs is typically ATR dependent and DNA-PKcs dependent. In short, γH2AX foci act as useful surrogate markers of DNA damage induced by these genotoxic agents.[111,115,121]

In the context of cancer therapy, quantitation of γH2AX is a particularly useful biomarker for evaluating the efficacy of radiation modifying compounds. For example, the radiation-sensitizing properties of histone deacetylase inhibitors (HDACi) have been widely investigated using γH2AX foci as an end point.[91,122–124] HDACi have emerged as a new class of anti-cancer therapeutics, with the hydroxamate, suberoylanilide hydroxamic acid (SAHA, vorinostat) already being approved by the US Food and Drug administration for the treatment of

cutaneous T-cell lymphoma.[125] The mechanism of action of this class of compounds involves, at least in part, inhibition of the activity of HDAC enzymes resulting in histone hyperacetylation, which in turns results in chromatin relaxation and altered gene expression.[126,127] Furthermore, the anti-cancer effects of HDACi, which include cell death and apoptosis, are due to interaction of the compounds with non-histone target proteins such as p53, tubulin and heat shock proteins.[126,127]

Although they have clinical potential as standalone therapeutics in cancer, HDACi have also been shown to modulate the effects of conventional chemotherapeutics and ionizing radiation. For example, both chromatin immunoprecipitation and immunofluoresence studies have been used to demonstrate increased radiation sensitivity of cancer cell lines pretreated with the HDACi, Trichostatin A (TSA) and valproic acid before irradiation.[91,122,123] Incidentally, the chromatin immunoprecipitation experiments using γH2AX as a molecular marker of DSBs was an early study identifying that heterochromatins (satellite 2 juxtacentromeric and α-satellite centromeric sequences) are resistant to γH2AX formation, compared to euchromatic loci (serum albumin, glyceraldehydes-3-phosphate dehydrogenase).[91] The utility of γH2AX foci in evaluating the radiation modifying properties of compounds such HDACi is highlighted by a recent study investigating valproic acid.[124] In this particular study, retention of γH2AX foci after exposure of cells to ionizing radiation was used to evaluate post-irradiation sensitization of the HDACi.[124]

It is noteworthy that quantitation of H2AX has also been used to investigate the HDACi-mediated (TSA, SAHA, MS-275, and OSU-HDAC42) enhancement of cell killing by the DSB-inducing agents bleomycin, doxorubicin and etoposide in cancer cells.[128] Similarly, evaluation of γH2AX foci was used to investigate the anti-leukaemic activity of combinations of the anthracycline, idarubicin, with the HDACi, SAHA and valproic acid, in cancer cells.[129] In another recent study, γH2AX was used as a marker to evaluate the combinatorial effects of the hydroxamic acid containing HDACi, RC307 and the synthetic atypical retinoid in ST1926 ovarian carcinoma cells.[130] Interestingly, γH2AX has also been applied as a marker to evaluate a relatively new combinatorial therapeutic strategy referred to as combination epigenetic therapy. This approach involves the simultaneous use of compounds that modify different epigenetic targets. For example, combinations of DNA methyltransferase (DNMT) inhibitors and HDACi are the most advanced and have demonstrated anti-cancer effects, particularly in patients with haematologic malignancies. In a recent clinical trial, γH2AX was quantitated in peripheral blood samples to evaluate the efficacy of combinations of the DNMT inhibitor, 5-azacytidine, and the HDACi, entinostat, in patients with myelodysplastic syndrome or acute myeloid leukaemia.[131]

Finally, environmental stressors such as tobacco smoke are potentially carcinogenic as they are potent inducers of DSBs.[132] γH2AX analysis has been applied in the investigation of the levels of induced genotoxicity caused by environmental carcinogens such as those present in cigarette smoke.[132,133] As an early cellular response to damage, γH2AX formation indicates the initiation of the DDR making it a suitable biomarker for detecting various forms of genotoxic insults.[68,132]

Conclusion

Analysis of γH2AX foci is currently a widely applied and robust method of investigating DNA damage and repair with many applications in the medical field. At present, the kinetics of γH2AX formation is well described in the context of X- and γ-ray radiation-induced DSBs. However, there are still gaps in our knowledge surrounding its distribution, spreading and disappearance, which will be undoubtedly addressed by future research. Further, it will be interesting to investigate the effects of other qualities of radiation and different types of genotoxic insults on γH2AX formation to gain a better understanding of the characteristics of this molecular marker. Quantitation of γH2AX foci is already being heralded as an accurate radiation biodosimeter and as a potential biomarker of aging and cancer. Given its specificity and sensitivity, analysis of γH2AX foci may well be adapted to numerous other medical uses, and the utility of this molecular marker for evaluating the efficacy of developmental therapeutics is a particularly exciting prospect.

Conflict of interest

The authors declare no conflict of interest.

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